

Application Number: 09/993,080
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Listing of Claims:

1. (Currently Amended) A method for producing a maize cell in which a nucleotide of interest is stably integrated, said method comprising:
 - (a) obtaining at least one immature embryo from a maize ear; and
 - (b) introducing said nucleotide construct into at least one cell of said immature embryo by microprojectile bombardment within 24 6 hours of obtaining said immature embryo.
2. (Original) The method of claim 1, further comprising contacting said immature embryo with an auxin-depleted a transformation support medium prior to said bombardment.
3. (Withdrawn) The method of claim 1 wherein said immature embryo is obtained about 6 days to about 14 days after pollination.
4. (Previously Presented) The method of claim 2 wherein said auxin-depleted transformation support medium comprises an osmotic potential greater than that produced by a medium containing 3% (w/v) sucrose.
5. (Original) The method of claim 4 wherein said auxin-depleted transformation support medium comprises an osmoticum consisting of sucrose, sorbitol, mannitol, polyethylene glycol, or combinations thereof.
6. (Original) The method of claim 2 wherein said auxin-depleted transformation support medium is phytohormone depleted.

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7. (Withdrawn) The method of claim 1 wherein said microprojectile bombardment comprises low-velocity impact of at least one microprojectile with said immature embryo.
8. (Withdrawn) The method of claim 7 wherein said microprojectile bombardment further comprises a gas pressure acceleration system comprising a rupture disk with a rupture disk rating that is at or below about 500 psi.
9. (Withdrawn) The method of claim 8 wherein said rupture disk rating comprises 100, 150, 200, 250, 300, 350, 400, 450 or 500 psi.
10. (Withdrawn) The method of claim 7 further comprising positioning said immature embryo between about 5 cm and about 12 cm from the macrocarrier platform.
11. (Cancelled)
12. (Currently Amended) The method of claim ~~14~~ 2 wherein said immature embryo is held on said auxin-depleted transformation support medium not more than about 4 hours before said nucleotide construct is introduced.
13. (Original) The method of claim 12 wherein said Immature embryo is held on said auxin-depleted transformation support medium not more than about 2 hours before said nucleotide construct is introduced.
14. (Withdrawn) The method of claim 1 wherein the genotype of said immature embryo is Hi-II or a hybrid from a cross of Hi-II with a second genotype.

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15. (Withdrawn) The method of claim 14 wherein said second genotype is PHN46, PHTE4, PHAA0, PHP18, PH05F, PH09B, PHP02, PHJ90, PH24E, PHT05, ASKC27 or PH21T.
16. (Withdrawn) The method of claim 1 further comprising regenerating said cell into a stably transformed maize plant.
17. (Withdrawn) The method of claim 16 wherein said cell comprises stably incorporated in its genome at least one copy of said nucleotide construct or part thereof.
18. (Withdrawn) The method of claim 16 wherein said nucleotide construct comprises a selectable marker gene, a marker gene or a cell cycle gene.
19. (Withdrawn) The method of claim 18 wherein said selectable marker gene is bar, nptII, hpt, the moCAH gene, herbicide resistance genes or antibiotic resistance genes.
20. (Withdrawn) The method of claim 18 wherein said regenerating comprises selection for the expression of said selectable marker gene.
21. (Withdrawn) The method of claim 16 where said nucleotide construct further comprises at least one nucleotide sequence of interest.
22. (Withdrawn) The method of claim 16 wherein said regenerating comprises inducing somatic embryogenesis.

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23. (Withdrawn) The method of claim 22 wherein said inducing somatic embryogenesis comprises providing said cell with an effective amount of an auxin.
24. (Withdrawn) The method of claim 23 wherein said auxin comprises 2,4-dichlorophenoxyacetate (2,4-D), indoleacetic acid (IAA), 3-indolebutyric acid (IBA), α -naphthaleneacetic acid (NAA), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), (4-chloro-2-methylphenoxy)acetic acid (MCPA), dicamba, chloramben or combinations thereof.
25. (Currently Amended) A method for producing a transgenic maize plant, said method comprising:
- (a) obtaining at least one immature embryo from a maize plant;
 - (b) introducing a nucleotide construct into at least one cell of said immature embryo by microprojectile bombardment wherein introduction of the nucleotide construct occurs within 6 hours of obtaining said immature embryo; and
 - (c) regenerating said cell into a transgenic maize plant wherein said cell comprises stably incorporated in its genome at least one copy of said nucleotide construct or part thereof.
26. (Original) The method of claim 25, further comprising contacting said immature embryo with an auxin-depleted a transformation support medium prior to said bombardment.
27. (Withdrawn) The method of claim 25 wherein said immature embryo is obtained about 6 days to about 14 days after pollination.

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28. (Previously Presented) The method of claim 26 wherein said auxin-depleted transformation support medium comprises an osmotic potential greater than that produced by a medium containing 3% (w/v) sucrose.
29. (Original) The method of claim 28 wherein said auxin-depleted transformation support medium comprises an osmoticum of sucrose, sorbitol, mannitol, polyethylene glycol, or combinations thereof.
30. (Original) The method of claim 26 wherein said auxin-depleted transformation support medium is phytohormone depleted.
31. (Withdrawn) The method of claim 25 wherein said microprojectile bombardment comprises low-velocity impact of at least one microprojectile with said immature embryo.
32. (Withdrawn) The method of claim 31 wherein said microprojectile bombardment further comprises a gas pressure acceleration system comprising a rupture disk with a rupture disk rating that is at or below about 500 psi.
33. (Withdrawn) The method of claim 32 wherein said rupture disk rating comprises 100, 150, 200, 250, 300, 350, 400, 450 or 500 psi.
34. (Withdrawn) The method of claim 31 further comprising positioning said immature embryo between about 5 cm and about 12 cm from the macrocarrier platform.
35. (Cancelled)

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36. (Currently Amended) The method of claim 35 26 wherein said immature embryo is held on said auxin-depleted transformation support medium not more than about 4 hours before said nucleotide construct is introduced.
37. (Original) The method of claim 36 wherein said immature embryo is held on said auxin-depleted transformation support medium not more than about 2 hours before said nucleotide construct is introduced.
38. (Withdrawn) The method of claim 25 wherein the genotype of said immature embryo is Hi-II or a hybrid from a cross of Hi-II with a second genotype.
39. (Withdrawn) The method of claim 38 wherein said second genotype is PHN46, PHTE4, PHAA0, PHP18, PH05F, PH09B, PHP02, PHJ90, PH24E, PHT05, ASKC27 or PH21T.
40. (Withdrawn) The method of claim 25 wherein said nucleotide construct comprises a selectable marker gene, a marker gene or a cell cycle gene.
41. (Withdrawn) The method of claim 40 wherein said marker gene comprises a selectable marker gene.
42. (Withdrawn) The method of claim 41 wherein said selectable marker gene is bar, nptII, hpt, the moCAH gene, herbicide resistance genes or antibiotic resistance genes.
43. (Withdrawn) The method of claim 40 wherein said marker gene comprises GFP.

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44. (Withdrawn) The method of claim 40 wherein said regenerating comprises selection for the expression of said selectable marker gene.
45. (Withdrawn) The method of claim 40 where said nucleotide construct further comprises at least one nucleotide sequence of interest.
46. (Withdrawn) The method of claim 25 wherein said regenerating comprises inducing somatic embryogenesis.
47. (Withdrawn) The method of claim 46 wherein said somatic embryogenesis comprises providing said cell with an effective amount of an auxin.
48. (Withdrawn) The method of claim 47 wherein said auxin comprises 2,4-dichlorophenoxyacetate (2,4-D), indoleacetic acid (IAA), 3-indolebutyric acid (IBA), α -naphthaleneacetic acid (NAA), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), (4-chloro-2-methylphenoxy)acetic acid (MCPA), dicamba, chloramben or combinations thereof.
49. (Currently Amended) A method for introducing a nucleotide construct into at least one cell within a ~~twenty-four~~ 6 hour period of time comprising:
 - (a) obtaining at least one immature embryo from a maize plant; and
 - (b) introducing a nucleotide construct into at least one cell of said immature embryo by microprojectile bombardment.
50. (Cancelled)
51. (Withdrawn) The method of Claim 49 further comprising regenerating said cell into a transgenic maize plant wherein said cell comprises stably incorporated in its genome at least one copy of said nucleotide construct or part thereof.

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52. (Cancelled)

53. (Currently Amended) The method of Claim 52 49 wherein said immature embryo is held on said auxin-depleted transformation support medium for not more than about 4 hours before said nucleotide construct is introduced.

54. (Currently Amended) The method of Claim 50 49 wherein the medium comprises an osmotic potential greater than that produced by a medium containing 3% (w/v) sucrose.

55. (Currently Amended) A method for high frequency stable transformation of freshly excised embryos said method comprising:

- (a) obtaining at least one immature embryo from a maize plant;
- (b) introducing a nucleotide construct into at least one cell of said immature embryo by microprojectile bombardment comprising 0.6 μ Au particles, rupture disk rating of about 200 p.s.i., and positioning said immature embryo between about 8 cm and about 12 cm from the macrocarrier platform, wherein said introduction occurs within 6 hours of excising said immature embryo.

56-61. (Cancelled)

62. (Withdrawn) The method of Claim 58 further comprising regenerating said cell into a transgenic maize plant wherein said cell comprises stably incorporated in its genome at least one copy of said nucleotide construct or part thereof.